

## SEQUENCE LISTING

<110> Vreeland, Valerie  
The Regents of the University of California

<120> Recombinant Minimal Catalytic Vanadium Haloperoxidases  
and Their Uses

<130> 023070-087110US

<140> US 09/596,794

<141> 2000-06-19

<150> US 09/151,189

<151> 1998-09-10

<160> 20

<170> PatentIn Ver. 2.1

<210> 1

<211> 2931

<212> DNA

<213> Fucus distichus

<220>

<221> CDS

<222> (228)..(2258)

<223> vanadium bromoperoxidase

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ttgtactgcg ccgcggttgcc aaaaaccgca actttaaaca gcgctcgca gcgccacatg 180

cttcccacgc atccacaaaa tcgacagtgg tategctgag cttgaat atg ctt tgc 236

Met Leu Cys

1

cat gca gcg gac acg aca aga ggc tct cct atg cct gac acc gga gtg 284

His Ala Ala Asp Thr Thr Arg Gly Ser Pro Met Pro Asp Thr Gly Val

5 10 15

ctt cgg ttg ctc aca tca gag cag cgc gct aaa ggt tgg aga cgc cag 332

Leu Arg Leu Leu Thr Ser Glu Gln Arg Ala Lys Gly Trp Arg Arg Gln

20 25 30 35

tta gag ggg gag aaa tca cta ggt ttt cat cca agc gag acg cct tat 380

Leu Glu Gly Glu Lys Ser Leu Gly Phe His Pro Ser Glu Thr Pro Tyr

40 45 50

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Ile Lys Tyr Leu Glu Gly Ser Glu Thr Trp Lys Lys Val Lys Leu Pro

55 60 65

acg gac ggc ata tcg gct tcc aag atc ctg ggt aaa att atg gcc agg 476

Thr Asp Gly Ile Ser Ala Ser Lys Ile Leu Gly Lys Ile Met Ala Arg

70 75 80

gtc cgc atc gct acc gcc ttg gct gtg gta ctg gcc gca ccc tgt ttg	524
Val Arg Ile Ala Thr Ala Leu Ala Val Val Leu Ala Ala Pro Cys Leu	
85 90 95	
gca ttc gac gag gtc aca gcc agt ggt gtt ttc cct gag gaa cac aag	572
Ala Phe Asp Glu Val Thr Ala Ser Gly Val Phe Pro Glu Glu His Lys	
100 105 110 115	
cac acc ggg gag gga aga cac ctc cag acc tgt aca aac tcc gac gat	620
His Thr Gly Glu Gly Arg His Leu Gln Thr Cys Thr Asn Ser Asp Asp	
120 125 130	
gcg ctg gat ccg acg gcg ccg aat aga agg gac aac gta gct ttt gcg	668
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135 140 145	
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Ser Arg Arg Asp Ala Ala Arg Arg Glu Arg Asp Gly Thr Gly Thr Val	
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Cys Gln Ile Thr Asn Gly Glu Thr Asp Leu Ala Thr Met Phe His Lys	
165 170 175	
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Ser Leu Pro His Asp Glu Leu Gly Gln Val Thr Ala Asp Asp Phe Ala	
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Val Pro Ala Gly Asp Pro Ala Gly Arg Leu Val Asn Pro Thr Ala Ala	
215 220 225	
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Phe Ala Ile Asp Ile Ser Gly Pro Ala Phe Ser Ala Thr Thr Ile Pro	
230 235 240	
ccg gta cct acc ctt tcc tct cct gag ctc gcc gct cag ttg gcg gag	1004
Pro Val Pro Thr Leu Ser Ser Pro Glu Leu Ala Ala Gln Leu Ala Glu	
245 250 255	
cta tac tgg atg gcg ctg gcc agg gat gta ccc ttt atg cag tat ggc	1052
Leu Tyr Trp Met Ala Leu Ala Arg Asp Val Pro Phe Met Gln Tyr Gly	
260 265 270 275	
acc gac gaa att acc act acc gcg gca gcc aac ctc gct gga atg gga	1100
Thr Asp Glu Ile Thr Thr Thr Ala Ala Ala Asn Leu Ala Gly Met Gly	
280 285 290	
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Gly Phe Pro Asn Leu Asp Ala Val Ser Ile Gly Ser Asp Gly Thr Val	
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Asp Pro Phe Ser Gln Leu Phe Arg Ala Thr Phe Val Gly Val Glu Thr	
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att acg gtc gaa ccg aag cag gag aca ttc gcc ccc gac ttg aac tat Ile Thr Val Glu Pro Lys Gln Glu Thr Phe Ala Pro Asp Leu Asn Tyr 340 345 350 355	1292
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gcc ggc ccc gaa gag tta gac gaa gag ctg cgt ttt atc cgt aac gcc Ala Gly Pro Glu Glu Leu Asp Glu Glu Leu Arg Phe Ile Arg Asn Ala 375 380 385	1388
cgc gac ctg gcc agg gtc tcc ttc gtg gac aat atc aac acc gaa gct Arg Asp Leu Ala Arg Val Ser Phe Val Asp Asn Ile Asn Thr Glu Ala 390 395 400	1436
tat cgc ggg tct ctt atc cta ctt gag ctg gga gcc ttc agc agg ccc Tyr Arg Gly Ser Leu Ile Leu Leu Glu Leu Gly Ala Phe Ser Arg Pro 405 410 415	1484
ggt atc aac ggt cca ttc atc gac agt gat cgg cag gcg ggc ttc gtc Gly Ile Asn Gly Pro Phe Ile Asp Ser Asp Arg Gln Ala Gly Phe Val 420 425 430 435	1532
aac ttc ggc acg tct cac tac ttc aga ttg ata ggt gcc gcc gag ctg Asn Phe Gly Thr Ser His Tyr Phe Arg Leu Ile Gly Ala Ala Glu Leu 440 445 450	1580
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cgc ccc gag gct ctc ggg ggt acc ctc cac aac acc atc gcg ggg gat Arg Pro Glu Ala Leu Gly Gly Thr Leu His Asn Thr Ile Ala Gly Asp 470 475 480	1676
cta gat gca gac ttc gac atc tcc ctt ctt gaa aat gat gag ctc ttg Leu Asp Ala Asp Phe Asp Ile Ser Leu Leu Glu Asn Asp Glu Leu Leu 485 490 495	1724
aaa cgt gtg gcg gag ata aat gcg gcg cag aat ccc aac aac gag gtc Lys Arg Val Ala Glu Ile Asn Ala Ala Gln Asn Pro Asn Asn Glu Val 500 505 510 515	1772
acc tac ctt ctt cca caa gct atc caa gtg gga tcg cca acg cac cct Thr Tyr Leu Leu Pro Gln Ala Ile Gln Val Gly Ser Pro Thr His Pro 520 525 530	1820
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Pro Val Phe Pro Ser Asp Asp Gly Leu Glu Leu Ile Asn Phe Glu Gly
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gca tgc ctt aca tat gag gga gag atc aac aag ctc gcg gtc aac gtc 2012
Ala Cys Leu Thr Tyr Glu Gly Glu Ile Asn Lys Leu Ala Val Asn Val
580 585 590 595

gca ttt ggg agg cag atg ctg ggc atc cac tat cgg ttc gac ggt atc 2060
Ala Phe Gly Arg Gln Met Leu Gly Ile His Tyr Arg Phe Asp Gly Ile
600 605 610

caa ggc cta ctt ctc gga gag aca atc act gta cga aca ctt cac cag 2108
Gln Gly Leu Leu Leu Gly Glu Thr Ile Thr Val Arg Thr Leu His Gln
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Glu Leu Met Thr Phe Ala Glu Glu Ala Thr Phe Glu Phe Arg Leu Phe
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acc gga gag gtc atc aaa ctt ttc cag gac ggg aca ttc tcc atc gat 2204
Thr Gly Glu Val Ile Lys Leu Phe Gln Asp Gly Thr Phe Ser Ile Asp
645 650 655

gga gat atg tgt tcc ggt ttg gtt tac act ggc gtg gcg gac tgc cag 2252
Gly Asp Met Cys Ser Gly Leu Val Tyr Thr Gly Val Ala Asp Cys Gln
660 665 670 675

gct tagtgcagaa aataataatt gtcg gatgct taaaatgcac ccacgaccaa 2305
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<213> Fucus distichus

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Lys Leu Pro Thr Asp Gly Ile Ser Ala Ser Lys Ile Leu Gly Lys Ile
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Met Ala Arg Val Arg Ile Ala Thr Ala Leu Ala Val Val Leu Ala Ala
          85             90             95

Pro Cys Leu Ala Phe Asp Glu Val Thr Ala Ser Gly Val Phe Pro Glu
          100            105            110

Glu His Lys His Thr Gly Glu Gly Arg His Leu Gln Thr Cys Thr Asn
          115            120            125

Ser Asp Asp Ala Leu Asp Pro Thr Ala Pro Asn Arg Arg Asp Asn Val
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Ala Phe Ala Ser Arg Arg Asp Ala Ala Arg Arg Glu Arg Asp Gly Thr
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Gly Thr Val Cys Gln Ile Thr Asn Gly Glu Thr Asp Leu Ala Thr Met
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Phe His Lys Ser Leu Pro His Asp Glu Leu Gly Gln Val Thr Ala Asp
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Asp Phe Ala Ile Leu Glu Asp Cys Ile Leu Asn Gly Asp Phe Ser Ile
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Cys Glu Asp Val Pro Ala Gly Asp Pro Ala Gly Arg Leu Val Asn Pro
          210            215            220

Thr Ala Ala Phe Ala Ile Asp Ile Ser Gly Pro Ala Phe Ser Ala Thr
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Thr Ile Pro Pro Val Pro Thr Leu Ser Ser Pro Glu Leu Ala Ala Gln
          245            250            255

Leu Ala Glu Leu Tyr Trp Met Ala Leu Ala Arg Asp Val Pro Phe Met
          260            265            270

Gln Tyr Gly Thr Asp Glu Ile Thr Thr Thr Ala Ala Ala Asn Leu Ala
          275            280            285

Gly Met Gly Gly Phe Pro Asn Leu Asp Ala Val Ser Ile Gly Ser Asp
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Gly Thr Val Asp Pro Phe Ser Gln Leu Phe Arg Ala Thr Phe Val Gly
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 Leu Asn Tyr Met Val Asp Phe Asp Glu Trp Leu Asn Ile Gln Asn Gly  
 355 360 365  
 Gly Pro Pro Ala Gly Pro Glu Glu Leu Asp Glu Glu Leu Arg Phe Ile  
 370 375 380  
 Arg Asn Ala Arg Asp Leu Ala Arg Val Ser Phe Val Asp Asn Ile Asn  
 385 390 395 400  
 Thr Glu Ala Tyr Arg Gly Ser Leu Ile Leu Leu Glu Leu Gly Ala Phe  
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 Ser Arg Pro Gly Ile Asn Gly Pro Phe Ile Asp Ser Asp Arg Gln Ala  
 420 425 430  
 Gly Phe Val Asn Phe Gly Thr Ser His Tyr Phe Arg Leu Ile Gly Ala  
 435 440 445  
 Ala Glu Leu Ala Gln Arg Ala Ser Cys Tyr Gln Lys Trp Gln Val His  
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 Arg Phe Ala Arg Pro Glu Ala Leu Gly Gly Thr Leu His Asn Thr Ile  
 465 470 475 480  
 Ala Gly Asp Leu Asp Ala Asp Phe Asp Ile Ser Leu Leu Glu Asn Asp  
 485 490 495  
 Glu Leu Leu Lys Arg Val Ala Glu Ile Asn Ala Ala Gln Asn Pro Asn  
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 Phe Pro Asn Pro Val Phe Pro Ser Asp Asp Gly Leu Glu Leu Ile Asn  
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 625 630 635 640

Arg Leu Phe Thr Gly Glu Val Ile Lys Leu Phe Gln Asp Gly Thr Phe  
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Asp Cys Gln Ala  
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 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:probe for  
           second conserved region between Curvularia and  
           Ascophyllum vanadium peroxidase active sites

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<210> 4  
 <211> 51  
 <212> DNA  
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<220>  
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           Ascophyllum vanadium peroxidase active sites

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 <211> 34  
 <212> DNA  
 <213> Artificial Sequence

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 <212> DNA  
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<212> DNA  
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<220>  
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primer for short construct

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<210> 8  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:Fucus  
peroxidase ligation independent cloning (LIC) 3'  
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<220>  
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1st conserved motif

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<210> 10  
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<213> Artificial Sequence

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vanadium-binding region 2, amino acids 528-546,  
2nd conserved motif



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Phe Ala Thr

<210> 11  
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<220>  
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<210> 12  
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 <222> (1)..(22)  
 <223> Xaa = any amino acid

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<210> 13  
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 <212> PRT  
 <213> Artificial Sequence

<220>  
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 <223> Xaa = any amino acid

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<220>
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<222> (12)
<223> Xaa = any amino acid, present in algal enzymes only

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<223> Xaa = any amino acid

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      motif from algal and fungal haloperoxidases

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<210> 16  
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 phosphatases and other proteins

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 <222> (1)  
 <223> Ser conserved in some proteins

<220>  
 <221> MOD\_RES  
 <222> (2)  
 <223> Tyr conserved in some proteins

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<220>  
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His Tyr Xaa Xaa Asp  
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<210> 18  
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<220>  
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 motif from algal and fungal haloperoxidases

<220>  
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<223> Description of Artificial Sequence:3rd conserved
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<223> Gly conserved in some proteins
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<220>
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<222> (10)
<223> Asp conserved in some proteins
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